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TG-repeat length polymorphism in the 5'-noncoding region of the growth hormone receptor gene in cattle and its association with meat production traits*

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A variable TG-repeat polymorphism was studied in the bovine growth hormone receptor (GHR) gene 5'-noncoding region. A total of seven alleles of the GHR gene were detected in six breeds. The length of the PCR-amplified variable region was from 306 to 320 bp, corresponding to 14 to 21 TG-repeats. Three additional alleles not reported so far, with 14, 19, and 21 TG-repeats were found. The associations between TG microsatellite genotypes and live body weight, daily live weight gain and carcass traits were studied in bulls of five beef breeds and Polish Black-and-White (Friesian) dairy cattle. In the latter the TG-repeat polymorphism in the 5'-noncoding region of the bovine gene *GHR* was significantly associated with mean body live weight, daily live weight gain, cold carcass weight and weight of lean and fat in valuable cuts. The longest 320-bp allele, with 21 TG repeats, was superior for most carcass traits, but when the growth rate was considered, the 320-bp allele homozygotes proved inferior to others.

KEY WORDS: cattle / carcass / fattening / gene / GHR / polymorphism

There is a great interest in using growth hormone (GH) to improve production traits in farm animals. Moreover, the gene encoding for GH and other genes related to "somatotropic axis" are considered promising candidate markers for selection purposes in animal breeding programmes [Parmentier *et al*.1999].

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GH acts on target cells through the GH receptor – GHR [Burton *et al*. 1994, Etherton and Bauman 1998]. The GHR is a member of cytokine/hematopoietin receptors superfamily. In most mammalian species, the gene coding for GHR consists of 9 exons (from 2 to 10) in the translated part and of a long 5'-noncoding region that includes several alternative untranslated exons, of which only exons 1A, 1B, and 1C have been studied in detail in bovine GHR gene [Jiang and Lucy 2001]. Distinct promoters regulate transcription from each of the alternative exons. The P1 promoter, which regulates the GHR expression in the liver, is associated with the exon 1A in cattle and sheep [Jiang *et al*. 1999].

It was shown that some productive traits of cattle, e.g. milk yield and composition were associated with polymorphism of GHR gene [Falaki *et al*. 1996, Aggrey *et al*. 1999, Blott *et al*. 2003]. Lucy *et al*. [1998] found the length polymorphism in a TG-repeat (microsatellite) in the P1 promoter located 86 bp upstream from the start site of the exon 1A in the bovine *GHR*. They found that an 11-TG-repeat allele commonly occurred in *Bos indicus* while alleles with 16 to 20 consecutive TGs are most common in Bos *taurus* cattle. However, the short 11-TG-repeat allele was found at low frequency in European cattle, e.g. in Aberdeen Angus. An association was reported between the microsatellite marker and growth rates in Angus steers [Hale *et al*. 2000].

In the present work the length polymorphism of TG-repeat was studied in bulls representing five beef breeds and one dairy breed in a search for possible associations between this polymorphism and meat production traits.

Material and methods

Animals

The study was performed on 71 fattening bulls belonging to five beef breeds – Charolaise, Limousine, Aberdeen Angus, Hereford, Simmental, and on 70 Polish Black-and-White (Friesian) bulls with more than 80% of Holstein-Friesian (HF) blood.

Bulls of each beef breed were the randomly chosen progeny of 5-7 sires. The animals were born in beef herds, artificially reared on milk replacer, concentrate and hay and transferred to the Institute farm, Jastrzębiec, at the age of 6-7 months. After reaching the age of 9 months the animals were fed *ad libitum* a total mixed ration (TMR), consisting of 75% corn silage, 20% concentrates and 5% hay. From the beginning of 13th to the end of 14th month of life the bulls were subjected to the 60-day test period for feed intake and conversion. Over that time the individual intake was recorded daily and chemical composition of the TMR was analysed weekly. One kg TMR contained 451 g dry matter, 0.36 UFV feed units for maintenance and meat production (INRA) and 40.95 g PDI (INRA). The bulls were weighed monthly and slaughtered at the age of 15 months at the local abattoir.

Seventy Polish Black-and-White (Friesian) bulls originated from 11 Holstein sires. The number of progeny per sire varied from 2 to 13. The animals were born and housed at local station from birth to slaughter at day 348 of age. They were kept under

the standardized feeding regimen and fed a ration formulated according to age (corn silage, concentrates and hay).

Approximately 10 ml blood samples were taken from each animal to test tubes containing K_2EDTA by authorized veterinarian.

All experimental procedures involving the animals were approved by the Local Ethics Commission (permission No 67/2001).

The carcasses were chilled for 24 hours at 4°C. Right (cold) carcass-sides were obtained and measured, and their valuable cuts measured and dissected into lean, fat and bone. A total of 17 carcass indicators were analysed.

Determination of GHR polymorphism

For the *GHR* genotyping DNA was isolated from blood by the method of Kanai *et al.* (1994) and PCR amplified. The amplified DNA extends from position -319 to -2 within the *GHR* promoter P1. For the analysis of TG microsatellite length the primers were based on the bovine *GHR* gene sequences (GenBank; AF126288 and U15731), as follows:

GHR-F – 5'-CTGGCGTATGGTCTTTGTCA-3' (Cy5 labelled).

GHR-R – 5'-TGGTCTTGCTGCTTTCCTAT-3'

Amplification was carried out for 35 cycles: 95°C for 20 s, 66°C for 30 s, and 72°C for 40 s. The fluorescent PCR products were separated in 6% denaturing polyacrylamide gels using an ALFexpress DNA Sequencer (AMERSHAM BIOSCIENCES Corp., Piscataway, NJ, USA). The PCR products were analysed after 5 min. denaturing in a 50% formamide solution containing blue dextran. In each lane 1 μl of PCR product was analysed together with a standard size marker. Results were visualized and the genotyping performed with the AlleleLinks 1.01 software (AMERSHAM BIOSCIENCES). After automated allele calling within Allele Links 1.01 programme, individual genotypes were checked by manual inspection before exporting the genotypes to Excel.

Statistical

The effects of microsatellite genotypes on the traits under study were analysed by the least-squares method as applied in the general linear model (GLM) procedure of SAS [1999-2001] as follows:

$$Y_{ijkl} = \mu + G_i + R_j + S_k + \beta(x_{ijkl} - x) + e_{ijkl}$$

where:

Y_{ijkl} – studied traits;

μ – overall mean;

G_i – fixed effect of microsatellites genotype in the 5'-noncoding region of the bovine growth hormone receptor gene (i=1...12);

R_j – fixed effect of a beef breed (j=1....5);

S_k – fixed effect of season at start of fattening (November-April and May-October, k=1,2);

$\beta(x_{ijkl} - x)$ – regression on the live body weight at the age of 8 months (Polish Friesian) or at the age of 9 months (beef bulls);

e_{ijkl} – the random residual effect.

Data for beef animals were analysed jointly and the effect of a breed has been included into statistical model.

The differences were evaluated with Duncan's test. The model includes all microsatellite genotypes in the 5'-noncoding region of the bovine *GHR*, but in Table 1 presented are only the estimates for genotypes found in three animals or more. The exact probability test was employed to evaluate possible deviations from Hardy-Weinberg equilibrium.

The differences between the observed and expected genotype frequencies within breeds and the observed genotype frequencies between breeds were estimated using the *chi* square test. Heterozygosity (H) was calculated according to Nei [1978] and polymorphism information content (PIC) according to Botstein *et al*. [1980].

Results and discussion

Genotype frequencies, mean H, and PIC of TG-repeat length polymorphism in the 5'-noncoding region of the bovine GHR gene in six cattle breeds are shown in Table 1. A total of seven TG-repeat alleles of the *GHR* were detected in 141 bulls belonging to the breeds tested. The length of these alleles ranged from 306 to 320 bp (Tab. 1). The 310-bp allele was found in Hereford only, the 306-bp only in Charolaise, and the 320-bp only in Polish Black-and-White (Friesian) bulls (Tab. 1). The 312-bp allele, most frequent in beef breeds, appeared rare (0.07) in Polish Black-and-White (Friesian) bulls (not shown in Tables). Among the seven alleles identified, six occurred both in homozygous and heterozygous systems; allele of 310-bp formed a homozygous genotype only, and was found exclusively in Herefords. Interestingly, heterozygous genotypes appeared only in Charolaise and Polish Black-and-White (Friesian) bulls. In bulls of remaining breeds the frequency of homozygous genotypes reached 100%. The mean coefficient H at this *locus* varied from 0.13 in Limousine to 0.68 in Charolaise (Tab. 1), while PIC from 0.11 in Limousine to 0.62 in Black-and-White bulls.

The associations were ascertained between TG microsatellite genotypes and body live weight, daily live weight gain and carcass traits. Although associations were calculated for both beef and dairy bulls, the results are given for Polish Black-and-White (Friesian) bulls and only for those traits for which differences between genotypes were proved (Tab. 2).

As shown in Table 2, the genotypes 314/314 and 314/318 of Black-and-White bulls were associated with significantly ($P \leq 0.05$) faster growth. From birth to slaughter the 314/314-genotype bulls gained 0.79 kg daily as compared to 0.75 kg gain of

Table 1. Number of animals and genotype frequencies (in brackets), mean heterozygosity (H), and polymorphic content (PIC) of TG-repeat length polymorphism in the 5'-noncoding region of the bovine growth hormone receptor (GHR) gene in six cattle breeds

Genotype	Black-and-White (n=70)*	Beef breeds					
		Charolaise (n=18)*	Limousine (n=16)*	Aberdeen Angus (n=10)*	Hereford (n=16)*	Simmental (n=11)*	Beef breeds total (n=71)*
306/306	–	1 (0.06)	–	–	–	–	1 (0.01)
306/312	–	1 (0.06)	–	–	–	–	1 (0.01)
306/314	–	2 (0.11)	–	–	–	–	2 (0.03)
310/310	–	–	–	–	1 (0.06)	–	1 (0.01)
312/312	3 (0.04)	8 (0.44)	15 (0.94)	7 (0.70)	10 (0.63)	10 (0.91)	50 (0.70)
312/318	4 (0.06)	–	–	–	–	–	–
314/314	24 (0.35)	4 (0.22)	1 (0.06)	2 (0.20)	5 (0.31)	1 (0.09)	13 (0.18)
314/318	11 (0.16)	2 (0.11)	–	–	–	–	2 (0.03)
314/320	13 (0.19)	–	–	–	–	–	–
316/316	3 (0.04)	–	–	1 (0.10)	–	–	1 (0.01)
316/320	2 (0.03)	–	–	–	–	–	–
318/318	4 (0.04)	–	–	–	–	–	–
320/320	6 (0.09)	–	–	–	–	–	–
H	0.67	0.68	0.13	0.51	0.54	0.18	0.45
PIC	0.62	0.58	0.11	0.41	0.43	0.15	0.40

*n – number of animals tested.

320/320 animals. Between 8th and 11th months of life the 314/314 and 314/318 bulls gained respectively 0.97 and 0.99 kg daily, significantly (P≤0.05) more than those of genotype 320/320 (0.83 kg). The 312/318 genotype was associated with the highest, and 314/318 with the lowest body live weight at the age of 8 months (224 *vs* 202 kg). The homozygous 320/320 genotype appeared favourable for the most important carcass traits. Cold carcass weight, weight of valuable cuts, and weight of lean were by 17.9 kg, 4.7 kg, and 3.7 kg higher, respectively, than those in the "worst" 318/318 genotype. The 314/318 genotype was significantly associated (P≤0.05) with high fat weight and fat content (%) in valuable cuts (by 1 kg and 1.5 per cent points more than in 314/320 bulls, respectively).

No significant associations were found between the TG-repeat polymorphism in the 5'-noncoding region of the bovine GHR gene and growth performance or carcass traits in beef cattle.

The TG-repeat occurs in homologous position of the *GHR* gene 5'-noncoding region in most mammalian species studied – human [Pekhletsky *et al*.1997], mouse [Menon *et al*. 1995], sheep [O'Mahoney *et al*. 1994], and cattle. It was found to be polymorphic in cattle [Lucy *et al*. 1998] and in European bison (our unpublished data).

Lucy *et al*. [1998] were first to report the TG-repeat polymorphism in the P1

Table 1. Overall least squares means (LSM) and their standard errors (SE) for Polish Black-and-White (Friesian) bulls' traits associated significantly with GHR gene TG microsatellite genotype

Trait	Genotype											
	[illegible] (n=[illegible])		[illegible] (n=[illegible])		[illegible] (n=[illegible])		[illegible] (n=[illegible])		[illegible] (n=[illegible])		[illegible] (n=[illegible])	
	LSM	SE	LSM	SE	LSM	SE	LSM	SE	LSM	SE	LSM	SE
Average daily gain from birth to slaughter (kg)	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
Average daily gain between [illegible] months	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
Live body weight at the age of [illegible] months (kg)	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
Cold carcass weight (kg)	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
Weight of valuable cuts in a carcass-side (kg)	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
Weight of lean in valuable cuts (kg)	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
Weight of bone in valuable cuts (kg)	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
Weight of fat in valuable cuts (kg)	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
Percentage of valuable cuts in a carcass-side	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
Per cent of lean in valuable cuts	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
Per cent of fat in valuable cuts	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]

[a,b...]Within rows means bearing the same superscript differ significantly at P≤[illegible]

promoter of the bovine GHR gene. Five alleles were found with variable TG-repeat number. The shorter allele, comprising 11 TG repeats was typical for *Bos indicus* cattle and was observed in Brahman and Nellore cattle, but later on it was also found with low frequency (0.05) in Angus cattle [Hale *et al*. 2000]. The authors suggested that the presence of 11-bp allele in Angus cattle is a possible result of "contamination" with *Bos indicus* genome. In the present study on *Bos taurus* cattle the presence of a short "indicine" allele was not confirmed, but the other four alleles reported by Lucy *et al*. [1998] were found. In the present study, the 310-bp, 312-bp, 314-bp, and 318-bp alleles correspond to the 16-, 17-, 18- and 20 TG-repeat alleles, respectively. Alleles with 17 and 18 TG-repeats appeared more frequent than others in all breeds, except for Polish Black-and-White, the result that corroborates the report by Lucy *et al* [1998]. The 312-bp allele, corresponding to 17 TG-repeats, appeared the most frequent in bulls of beef breeds, but the least frequent in Polish Black-and-White. In the latter, the most frequent was the 314-bp allele, corresponding to 18 TG-repeats. In this study three additional alleles with 14, 19, and 21 TG-repeats were found, not previously reported.

Although microsatellites are most often considered to be neutral markers, recent evidence suggests that poly-TG elements might have functional significance. They often adopt a left-handed double helical conformation of DNA, called Z-DNA that may be important in mutagenesis, recombination, and control of gene expression [Majewski and Ott 2000]. The length of the TG-repeats has been shown to affect gene transcription rates of several human genes, including those coding for EGF receptor, matrix metalloproteinase-9 enzyme, and type I collagen $\alpha 2$. In all cases increase in gene transcription rates was positively related to the length of the TG-repeat [Hadjiyannakis *et al*. 2001]. An association was reported between the microsatellite marker and growth rate in Angus steers by Hale *et al*. [2000]. Their results showed the superiority of genotypes with the long alleles for weight at weaning and carcass weight.

Our results confirmed the superiority of the longest 320-bp allele (21 TG-repeats) for carcass traits in dairy Black-and-White cattle, but when the growth rate is considered, the 320-bp allele proved inferior to others. However, it is worthwhile to note that the homozygous genotype with two 320-bp alleles – the one found superior for most carcass traits in Black-and-White (Friesian) cattle – was absent in bulls of all beef breeds considered. In most beef bulls genotypes with 312-bp and 314-bp alleles were found, and those were shown to have no effect on carcass traits in the studied group of Black-and-White cattle.

In summary, the results presented here showed that the TG-repeat polymorphism in the 5'-noncoding region of the bovine GHR gene was significantly associated with mean body live weight, mean body live weight gain, cold carcass weight, weight of lean and fat in valuable cuts. However, further studies, performed on bigger populations of cattle, are necessary to confirm this notion.

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Polimorfizm długości powtórzeń dinukleotydu TG w rejonie niekodującym 5' genu receptora hormonu wzrostu (GHR) bydła i jego związek z cechami produkcyjności mięsnej

S t r e s z c z e n i e

Badano polimorfizm długości powtórzeń dinukleotydu TG (mikrosatelity) w rejonie niekodującym 5' genu receptora hormonu wzrostu (GHR) bydła. Analizując próbki DNA pochodzące od sześciu ras bydła stwierdzono łącznie siedem alleli genu GHR. Amplifikowany metodą PCR fragment DNA miał od 306 do 320 pz długości, co odpowiada 14 do 21 powtórzeń TG. Wykryto trzy nowe allele z 14, 19 i 21 powtórzeniami TG, nie opisane poprzednio w literaturze. Zbadano związek pomiędzy polimorfizmem genu GHR a masą ciała, tempem wzrostu i cechami jakości tuszy bydła mięsnego i polskiego bydła czarno-białego (cb). Wykazano istotny statystycznie ($P \leq 0.05$) związek ze średnimi dziennymi przyrostami, masą ciała, masą schłodzonej tuszy i masą chudego mięsa i tłuszczu w wartościowych wyrębach tuszy u bydła cb. „Najdłuższy" allel 320-pz, zawierający 21 powtórzeń TG, okazał się korzystny dla większości cech jakości tuszy, jednak homozygoty tego allelu wykazywały najmniejsze tempo wzrostu.